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A VIRUS-IN-WATER STUDY OF FINISHED WATER FROM SIX COMMUNITIES

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Health Effects Research Laboratory
Cincinnati, Ohio

September 1975

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16. ABSTRACT

Fifty-six finished water study samples up to 1900 liters were collected and successfully processed for virus isolations from six communities. Eight additional samples were inoculated with low levels of virus as a check (positive control) on the sensitivity of our procedure. Six of the eight positive control viruses were recovered. Two virus serotypes were not recovered at an input level of one infectious unit per 19 liters. No viruses were isolated from the study samples. Bacteriological tests with experimental large volume procedures showed that coliform bacteria were present in 25 of 71 samples. The coliform densities were very low and in all cases were within the limits of the 1962 USPHS Drinking Water Standards.

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Program Element No. 1CA046

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. In Cincinnati, the Environmental Research Center possesses this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

The Health Effects Research Laboratory conducts studies to identify environmental contaminants singly or in combination, discern their relationships, and to detect, define, and quantify their health and economic effects utilizing appropriate clinical, epidemiological, toxicological, and socio-economic assessment methodologies.

Enteric viruses are an environmental contaminant through the sewage system. This continuing study was designed to determine if human enteric viruses could be found in treated drinking water and to field test virus concentrating equipment and procedures as they were improved by ongoing research in the Health Effects Research Laboratory.

R. J. Garner
Acting Director

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INTRODUCTION

Six sites in three States were selected for a virus-in-water study, with the assistance of EPA regional staff and State health department (or State EPA) personnel. The sites selected were: (1) Columbus, Ohio (Dublin Road Plant), (2) Sidney, Ohio, (3) Seymour, Indiana, (4) Muncie, Indiana (5) St. Joseph, Missouri and (6) Kansas City, Missouri. The primary criterion for selection of sites was based on a treatment plant's use of surface source water having domestic contamination as indicated by high fecal coliform counts. An effort was also made to select sites that used conventional flocculation or softening procedures. In addition, plants of varying sizes were chosen as indicated by water output volumes.

The study had a two fold purpose: (1) to determine if human enteric viruses could be detected in treated finished water with the equipment and procedures presently available, and (2) to field test virus-sampling equipment and procedures as they were improved by ongoing research in this laboratory.

MATERIALS AND METHODS

The types of treatment and the fecal coliform densities of the source waters at the six sites are shown in Table 1. Plant capacity ranged from 5,600 to 795,000 m^3 per day (1.5 to 210 million gallons per day) and treatment included both conventional alum flocculation and lime-soda softening. The range and geometric means of the fecal coliform densities in the source waters indicated that relatively heavy pollution was occurring intermittently at the Ohio and Indiana sites and almost constantly

at the Missouri sites.

A flow-through virus-adsorbent system was used throughout this study. Water samples were adjusted to pH 3.5 and in the initial samples, divalent cations were added to enhance virus adsorption (it was later shown that the addition of cations did not enhance virus recovery). Sodium thiosulfate was also added to neutralize residual chlorine in the water samples. After the desired volume of water had been processed, the virus adsorbant was then transported to the laboratory on ice and eluted with one liter of eluting fluid. A secondary concentration step was conducted to further reduce the sample (eluate) volume. The reconcentrated sample (10-30 ml) was then distributed equally among four cell types: (1) primary African green monkey kidney (AGMK), (2) primary human embryonic kidney (HEK), (3) continuous porcine kidney and (4) continuous monkey kidney (VERO). These inoculated cell monolayers were observed for cytopathic effects (CPE) for 14 days; if the results were negative, two consecutive blind passages were conducted before declaring a sample negative for viruses. Cell monolayers manifesting CPE were confirmed as viral CPE and the serotype identified by serum neutralization test.

Sampling was begun in November 1973 with the objective of collecting and processing 380 liters (100 gal) of water per sample. The first 11 samples were collected using a 0.45-μm porosity cellulose nitrate cylindrical filter (Millitube) as the virus adsorbent. The filter was eluted with 5x nutrient broth pH 9.0 and this eluate was reconcentrated by an aqueous polymer 2-phase procedure. Premature plugging, eluate leakage and other problems led to the discontinuation of this procedure. Laboratory experimentation had previously indicated that a stack of three

293-mm cellulose nitrate membrane disks (8, 1.2 and .45-μm porosities) was a better system. Beginning with sample number 12, this system replaced the Millitube as the primary adsorbent. In addition, the nutrient broth eluent was replaced with glycine buffer pH 11.5 (later reduced to pH 11.1) and the polymer 2-phase reconcentration procedure was replaced with a second virus adsorbent system--a stack of three small diameter (47 mm) epoxy-fiberglass-asbestos disks of 5, 1 and .45-μm porosity. Premature plugging occurred on occasions with the 293-mm membrane disk.

Further experimentation in our laboratory with an epoxy-fiberglass filter tube of 8.0-μm porosity had previously indicated that a larger sample volume (1,900 liters) could be collected with a greater poliovirus recovery sensitivity when three filter tubes in parallel were used as the virus adsorbent[1]. This unit also had advantages in size and ease of handling and was adopted in July 1974 as the primary virus adsorbent system. Premature plugging has not been experienced with this virus adsorbing filter.

A positive control system was included in this study to keep a continual check on the virus recovery capability of the procedure. Ampules of sterile nutrient broth were prepared in the laboratory and low levels of selected enteric virus serotypes were introduced into a number of these ampules. The ampules were then coded, randomized by number and the content of a single ampule was introduced into each field sample as it was being processed. Neither the sample collecting team nor the person "reading" the cell culture for CPE knew which ampules contained a virus. The project officer monitored the distribution of

the positive controls and also conducted the serological test that confirmed the recovery of a positive control when CPE was observed in the cell culture system. The selection of different virus serotypes as positive controls provided an opportunity to evaluate the virus detection efficiency of the procedure for representatives of the various subgroups within the enteric virus group.

Samples were collected to test for the presence of coliform bacteria at the time that water samples were taken for virus testing. This procedure was followed to attempt to relate any virus isolations to the presence of the coliform indicator of microbial pollution. The presence of coliform bacteria was determined by the membrane filter procedure with 0.1, 1.0 and 5.0 liters of sample and by two experimental procedures. The first experimental procedure was a modified MPN test in which membrane filters were used and up to 5.5 liters of water were examined; the other was a large-volume sampler (LVS) technique that utilized epoxy-fiberglass filter tubes whereby 380 liters of water were filtered and the coliform densities determined by the MPN procedure. The LVS was under development at the time of this study and was not available for field use until June 1974. The inclusion in this study of 1 and 5 liter sample volumes for the membrane filter procedure and the two experimental procedures reflected the concern that 100-ml standard portions may be insufficient volumes to examine if the coliform test is to be a satisfactory indicator of viral contamination of a water supply. If viruses had been isolated from the finished water samples taken in this study, an attempt would have been made to correlate these isolations with coliform densities derived from the three bacteriological procedures.

RESULTS

Between November 1973 and February 1975 seventy nine samples were collected from the six water treatment plants at a point after final treatment and disinfection. Pertinent data on the samples collected at each of the six sites are shown in Tables 2 through 7. Turbidities were generally very low (<1.0 unit) and free chlorine residuals were over 1.0 mg/l in most samples from all sites except Kansas City which added ammonia to produce chloramines. All samples were alkaline and approached pH 10 at the plants using a lime softening procedure (see Table 1). Difficulties were experienced in processing 15 of the 79 samples. The processing of these samples is recorded as unsatisfactory in the tables because it was felt that virus detection could have been significantly compromised. Of the remaining 64 samples, eight received the positive control viruses as a check on the virus recovery procedure. The control viruses were recovered in six of the eight samples (Table 8). Two virus serotypes (Reo 1 and Echo 27) were not recovered when introduced at a concentration of one infectious unit per 19 liters. Other viruses were recovered at this low level and studies are now underway to determine the cause of our failure to recover these two viruses. The remaining 56 samples represent the study samples from the six sites.

Cell culture changes indicative of viral replication were observed in AGMK cells that had received two of the study samples. These two isolates were subsequently identified, by an independent laboratory and confirmed in our laboratory, as a monkey papovavirus (SV 40) that is commonly indigenous to primary monkey kidney cells. None of the other

three cell types used manifested CPE. It was concluded that these two viral isolates were cell culture contaminants and that therefore, no viruses had been isolated from any of the study samples.

The source water quality at each of the six sites was very poor at certain times during the study period. At least one sample from each site exceeded the maximum fecal coliform densities recommended for source water[2]. The two Missouri sites used the Missouri River as their water source, a river that is well recognized as having a heavy microbial pollution load. Fecal coliform densities were consistently high at the intake of these two water treatment plants (Table 1).

Bacteriological tests for coliform organisms were conducted on 71 (37 with the LVS) of the 79 finished water samples. Coliform-group organisms or fecal coliforms were detected in 25 of these samples by at least one of the three procedures used (Table 9). Using the membrane filter procedure with up to 5 liters of sample, coliforms were detected in seven samples, one of which also yielded fecal coliforms. Using the modified MPN procedure with 5.5 liters of sample, coliforms were detected in 11 samples, two of which also yielded fecal coliforms. Using the LVS and examining 380 liters of water, coliforms were detected in 17 samples, eight of which also yielded fecal coliforms. In only three samples (all from Seymour) were coliforms detected by all three procedures. Since these samples were collected and partially processed in the field under somewhat adverse conditions, it is possible that some of the positive samples represent contamination. In all cases, the coliform densities

were extremely low and all samples met the 1962 U.S. Public Health Service Drinking Water Standard.

DISCUSSION

The health risk of human viruses in treated water supplies in this country has not been established. In fact, the studies by this laboratory are believed to be the first attempt to use a sensitive, large-volume virus recovery procedure to examine drinking water for the presence of viruses at plants that use domestically polluted surface water as a source. In the absence of background surveillance data, the volume of each sample, the frequency of sampling and the number of samples have not been established for determining the viral quality of a water supply. The virus recovery efficiency of our procedure had been evaluated in the laboratory with sample volumes only up to 1,900 liters (500 gal). Therefore, we made no attempt to exceed this volume with the field samples. The frequency of sampling and the total number of samples taken was primarily determined by staff and facilities available for the study and distance of the site from the laboratory. It was felt that more than one sample was required from each site, because of the fluctuations in the quality of source waters and the variations in the day to day operation of water treatment plants, before a definitive conclusion could be drawn as to the virological quality of the water.

In this study, three to 14 samples per site were collected and successfully processed. At this point in the study of the virus-in-water question, we believe that fewer than five samples are an insufficient

number to evaluate a water supply. Therefore, sampling will continue at the Kansas City site (4 satisfactory study samples collected) and the St. Joseph site (3 satisfactory study samples collected) before an evaluation will be made. Nine to 14 samples were successfully processed from each of the Ohio and Indiana sites. Analysis of the waters at the time of sampling indicated that treatment for removal of pathogenic microorganisms was very good, i.e., high free chlorine residuals and low turbidities. Therefore, in the absence of virus isolations, sampling at these four sites will not be continued as more appropriate sites are located.

The isolation of fecal coliforms from six of the Seymour, Indiana, samples with the LVS is of special interest (Table 9). The significance of low numbers (7 to > 43/1,000 liters) of these organisms in treated drinking water with free chlorine residuals >1.0 mg/l cannot be evaluated in the absence of virus isolations. Continued sampling with the LVS and a thorough evaluation of its recovery efficiency is certainly in order and will be carried out.

The results of this study of finished waters produced by conventional treatment methods from surface waters of a rather poor quality have shown that if viruses were present in these waters, the numbers were below the detectable level of a sensitive virus recovery procedure. Laboratory tests have shown that the sensitivity of the virus recovery procedures used in this study was such that poliovirus could consistently be detected at contamination levels of 3 to 5 units per 380 liters when 1,900 liters were sampled.[4] Through the evaluation of positive controls, field tests have recovered six other members of the enteric virus group at concentrations

of 100 to 1,000 virus units per 380 to 1,900 liters. Therefore, we assume that if viruses were present in the study samples, the concentration was below one virus unit per 3.8 liters (gal) of finished water. The fact that these water treatment systems were challenged by very poor source water on numerous occasions and yet produced a finished water in which no viruses could be recovered seems to confirm the adequacy of good conventional treatment for virus removal.

In light of the failure to recover virus from up to 1,900 liters (500 gal) of finished water from these sites, it now seems desirable to study water plants or water systems that have compromised or completely omitted conventional treatment of a source water known to have recently received domestic waste. We are presently seeking such sites. Documentation of the level of viral contamination in the source water of some sites may also be desirable as a basis for evaluating the viral removal efficiency of the treatment systems. Procedures for collecting and processing large volumes of untreated surface water for virus isolation are presently being evaluated in our laboratory.

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Table 1. TREATMENT PROCESSES EMPLOYED AND FECAL COLIFORM DENSITIES OF SOURCE WATER AT SIX STUDY SITES

Item	Treatment Site					
	Columbus	Sidney	Muncie	Seymour	Kansas City	St. Joseph
Treatment:						
Potassium permanganate			X[a]		X	
Prechlorination	X		X	X	X	
Presedimentation					X	
Ammonia					X	
Alum	X	X	X	X		X[a]
Lime	X		X	X[a]		
Carbon		X	X	X[a]	X[a]	X[a]
Polymer					X[a]	X
Lime-soda softening	X	X			X	
Flocculation	X	X	X	X	X	X
Sedimentation	X	X	X	X	X	X
Chlorination		X				X
Recarbonation	X	X			X	
Sand filtration	X	X	X	X	X	X
Post chlorination	X		X	X	X	X
Contact time (hr)	26	7	8	14	22	24
Capacity/mean output (10,000 m^3/day)	26.5/13.6	1.4/1.1	6.0/4.9	0.56/0.45	79.5/41.5	11/5.3
Fecal coliform MPN/100 ml of source water (range)	38-3,000	40-2,800	50-2,200	11-14,000	1,700-4,300	280-7,000
Geometric mean	280	263	210	536	3,010	1,890

[a] Added as needed

Table 2. VOLUME, WATER QUALITY DATA, AND PROCESSING RESULTS OF SAMPLES COLLECTED AT COLUMBUS, OHIO

Date collected	Volume collected (liters)	Turbidity (FTU)	Residual chlorine (mg/l)		pH	Result of sample processing[a]
			Free	Combined		
12/5/74	265	.08	2.6	.38	9.5	S
1/21/74	380	.49	1.5	.45	9.4	S
3/11/74	380	.07	1.5	.40	9.3	S
3/25/74	380	.09	1.4	.35	9.5	PC
4/16/74	380	.03	1.4	.20	9.2	S
5/1/74	380	.04	1.4	.15	9.6	S
5/15/74	380	.05	1.8	.00	9.8	S
5/29/74	380	.05	1.5	.32	10.1	S
6/26/74	380	.07	1.7	.40	9.6	S
7/10/74	1,900	.28	1.4	.27	9.6	S
8/14/74	1,900	.72	1.4	.20	10.2	S
9/10/74	1,900	.04	1.2	.20	9.9	S
10/2/74	1,900	.09	1.6	.20	10.1	S
10/16/74	1,900	.04	1.3	.10	10.2	S
11/6/74	1,900	.02	1.3	.40	10.1	S

[a] S, successful; PC, positive control.

Table 3. VOLUME, WATER QUALITY DATA, AND PROCESSING RESULTS OF SAMPLES COLLECTED AT SIDNEY, OHIO

Date collected	Volume collected (liters)	Turbidity (FTU)	Residual chlorine (mg/l)		pH	Result of sample processing[a]
			Free	Combined		
11/26/73	570	.00	2.0	1.1	9.0	US
1/16/74	380	.02	1.3	1.8	8.8	S
3/12/74	380	.03	1.4	0.6	9.3	S
3/26/74	380	.02	0.9	1.0	9.3	US
4/17/74	380	.03	1.3	0.7	8.8	S
4/30/74	380	.02	1.4	0.5	9.2	PC
5/14/74	380	.02	1.6	1.4	9.7	S
5/28/74	380	.01	2.2	0.5	9.7	S
6/25/74	380	.05	1.9	1.0	9.2	S
9/11/74	1,900	.00	1.3	0.4	9.7	S
10/1/74	1,900	.08	1.9	0.9	9.8	PC
10/15/74	1,900	.04	1.6	0.3	9.1	S
11/5/74	1,900	.05	1.4	0.8	9.9	PC

[a] S, successful; US, unsuccessful; PC, positive control.

Table 4. VOLUME, WATER QUALITY DATA, AND PROCESSING RESULTS OF SAMPLES COLLECTED AT MUNCIE, INDIANA

Date collected	Volume collected (liters)	Turbidity (FTU)	Residual chlorine (mg/l)		pH	Result of sample processing[a]
			Free	Combined		
12/18/73	285	0.26	1.2	0.1	7.7	S
1/14/74	380	0.04	1.8	0.3	7.4	US
2/26/74	380	0.76	1.6	0.3	7.1	S
3/19/74	380	0.41	1.9	0.5	7.4	S
4/10/74	380	0.28	1.5	0.4	7.3	S
4/24/74	285	0.14	2.0	0.2	7.4	US
5/8/74	170	0.51	1.0	0.4	7.8	US
5/22/74	380	0.10	1.6	0.2	7.6	S
6/5/74	380	0.08	1.5	0.1	7.7	S
7/3/74	1,900	0.18	1.0	0.3	7.2	S
7/17/74	1,900	0.36	1.4	0.3	8.4	S
7/24/74	1,900	0.11	1.7	0.3	8.2	S
9/4/74	1,900	0.33	1.4	0.3	8.5	S
9/26/74	1,900	0.56	1.3	0.1	8.9	S
10/9/74	1,900	0.71	2.0	0.2	9.2	US
10/31/74	1,900	1.00	1.1	0.2	8.1	S
11/12/74	1,900	0.30	1.7	0.3	8.4	S
12/3/74	1,900	0.56	2.2	0.1	7.7	PC

[a] S, successful; US, unsuccessful; PC, positive control.

Table 5. VOLUME, WATER QUALITY DATA, AND PROCESSING RESULTS OF SAMPLES COLLECTED AT SEYMOUR, INDIANA

Date collected	Volume collected (liters)	Turbidity (FTU)	Residual chlorine (mg/l)		pH	Result of sample processing[a]
			Free	Combined		
11/27/73	95	.60	0.4	0.2	7.3	S
1/6/74	240	.14	0.7	0.1	7.6	US
2/12/74	380	.36	1.0	0.3	7.6	S
3/18/74	380	.19	1.0	0.2	7.4	S
4/9/74	380	.43	0.7	0.3	7.2	S
4/23/74	380	.08	0.8	0.3	7.6	PC
5/7/74	380	.30	0.7	0.2	8.2	S
5/21/74	380	.34	0.4	0.2	7.5	S
6/4/74	380	.83	0.2	0.0	7.2	S
7/2/74	1,900	.11	0.4	0.5	7.2	US
7/16/74	1,900	.14	0.3	0.0	7.8	US
7/23/74	1,900[b]	.03	2.5	0.3	8.2	US
8/8/74	1,900	.03	1.7	0.3	7.9	S
9/5/74	1,900	.36	2.4	0.2	8.7	S
9/25/74	1,900	.08	1.5	0.3	9.1	S
10/8/74	1,900	.38	1.8	0.1	8.8	US
10/30/74	1,900	.40	1.6	0.2	8.0	US
11/11/74	1,900	.10	1.7	0.3	8.3	S
12/2/74	1,900	.44	1.9	0.1	7.3	S
1/4/75	1,900	1.50	1.7	0.4	7.0	US
1/15/75	1,900	1.00	2.0	0.1	7.1	S
2/12/75	1,900	.45	2.4	0.1	8.1	PC
2/13/75	1,900	.44	2.7	0.1	8.4	S

[a] S,successful; US, unsuccessful; PC, positive control.
[b] Began sampling at new site in plant.

Table 6. VOLUME, WATER QUALITY DATA, AND PROCESSING RESULTS OF SAMPLES COLLECTED AT KANSAS CITY, MISSOURI

Date collected	Volume collected (liters)	Turbidity (FTU)	Residual chlorine (mg/l)		pH	Result of sample processing[a]
			Free	Combined		
12/12/73	270	.05	.03	1.2	9.5	S
6/12/74	380	.07	.00	0.6	10.5	PC
7/31/74	1,140	.03	.02	0.8	10.2	US
8/21/74	1,900	.02	.00	1.0	9.7	S
9/18/74	880	.03	.00	1.0	9.9	S
11/20/74	1,900	.71	.00	0.8	9.8	S

[a] S, successful; US, unsuccessful; PC, positive control.

Table 7. VOLUME, WATER QUALITY DATA, AND PROCESSING RESULTS OF SAMPLES COLLECTED AT ST. JOSEPH, MISSOURI

Date collected	Volume collected (liters)	Turbidity (FTU)	Residual chlorine (mg/l)		pH	Result of sample processing[a]
			Free	Combined		
12/12/73	260	.28	2.0	0.1	7.9	S
6/12/74	380	.09	1.9	0.3	8.0	US
7/30/74	1,900	.63	1.7	0.3	8.0	S
8/20/74	1,900	.08	1.9	0.2	8.4	S

[a] S, successful; US, unsuccessful.

Table 8. VIRUS INOCULATED INTO RANDOMLY SELECTED SAMPLES AS A CONTROL ON THE VIRUS RECOVERY SENSITIVITY OF THE SAMPLING PROCEDURE

Sampling site	Sample volume (liters)	Date	Virus Input		Result of isolation procedure[b]
			Type	Amount[a]	
Columbus	380	3/25/74	Echo 24	100	R
Seymour	380	4/23/74	Coxsackie A21	1,000	R
Sidney	380	4/30/74	Adeno 15	100	R
Kansas City	380	6/12/74	Echo 13	100	R
Sidney	1,900	10/1/74	Reo 3	100	NR
Sidney	1,900	11/5/74	Coxsackie B6	100	R
Muncie	1,900	12/3/74	Echo 27	100	NR
Seymour	1,900	2/12/75	Polio 1	160	R

[a] The poliovirus was quantitated by the plaque method; all others by the tissue culture Infectious Dose 50% method.

[b] R, virus recovered; NR, virus not recovered

Table 9. POSITIVE COLIFORM/FECAL COLIFORM FINDINGS FOR FINISHED WATER SAMPLES FROM SIX STUDY SITES

Sample site and date collected	Membrane filter[a]		Modified MPN[b]		Large volume sampler[c]	
	Total coliforms	Fecal coliforms	Total coliforms	Fecal coliforms	Total coliforms	Fecal coliforms
Columbus:						
12/5/73	-	-	+	-	-	-
1/21/74	-	-	-	-	+	-
10/2/74	-	-	-	-	+	-
11/6/74	-	-	+	-	+	-
Sidney:						
9/11/74	+	+	+	+	-	-
10/8/74	-	-	+	-	-	-
11/5/74	-	-	-	-	+	-
Muncie:						
7/3/74	+	-	-	-	-	-
9/4/74	-	-	-	-	+	+
9/26/74	+	-	-	-	+	-
10/31/74	-	-	-	-	+	+
Seymour:						
7/23/74	-	-	+	-	-	-
9/5/74	-	-	-	-	+	+
9/25/74	-	-	-	-	+	+
10/8/74	-	-	-	-	+	+
10/30/74	+	-	+	+	+	+
11/11/74	+	-	+	-	+	+
12/2/74	+	-	+	-	+	+
1/14/75	-	-	-	-	+	-
1/15/75	-	-	-	-	+	-
2/12/75	-	-	+	-	+	-
2/13/75	-	-	-	-	+	-
Kansas City:						
12/12/73	-	-	+	-	-	-
6/12/74	-	-	+	-	ND[d]	ND
St. Joseph:						
6/12/74	+	-	-	-	ND	ND

[a] Up to 5 liters were tested; negative findings indicate a value <0.02; the largest positive value was 0.12 organism/100 ml.
[b] 5.5 liters were tested; negative findings indicate a value <0.02 organism/100 ml; the largest positive value was 0.23 organism/100 ml.
[c] 380 liters were tested; negative findings indicate a value <0.00059 organisms/100 ml; the largest positive value was >0.0043/100 ml.
[d] ND, not done.